NUCLEUS AND CYTOPLASM AS VEHICLES OF HEREDITY[1]

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THERE have been of late several attempts to effect a compromise between theories of heredity through the cytoplasm and theories which regard the chromosomes as the vehicles of inherited characters. Conklin ('08) was the first to suggest that egg, embryonic and general phyletic characters of any stage of the organism were determined in the egg cytoplasm while the determiners in the chromosomes made their presence known only through the specific or individual adult characters. Shull (1916) has elaborated this suggestion, and has brought to its support not only the older data on maternal inheritance, matrocline hybrids and the facts of development which relate to polarity, symmetry and organ-forming substances, but has added new evidence of his own from experiments with rotifers. Most recently, Loeb, in his book "The Organism as a Whole," has advanced a similar compromise theory, based on similar evidence.

Before examining in detail the experimental basis for such a compromise, it is important that the terms to be used be clearly and unmistakably defined. The first of these is the word "determined." That a character is determined in the germ cell means that the differential, causal antecedents of that character are present in the germ cell. It does not mean that the character itself is present in the germ in any form, but rather that it is represented by substances or forces which not only *stand for* the character but in some way bring about its expression.

[1] A review made at the suggestion of Dr. H. W. Rand to whom grateful acknowledgement is due.

If this definition of "determined" is accepted, two kinds of continuity in organisms are immediately differentiated. The first sort may be called *substantial* continuity. It is the carrying over from one generation to the next of autonomous organizations of protoplasm in a manner analogous to the carrying of the bacilli of certain diseases (*e. g.*, syphilis) in the germ cell. Here the germ cell is a passive vehicle. The character is present, not determined; and its changes from fertilized egg to adult are mere proliferations. If hereditary characters were to be so viewed, and the view carried to its logical conclusion, the result would be something very like an "emboitement" theory, which facts of development have proved to be untenable. Substantial continuity is hence only a concomitant rather than a part or a method of heredity.

The second type of continuity may be called "*genetic*" continuity, and characters which are genetically continuous are those which show a new coming into being with every generation. They are developed *anew*, and their resemblance to homologous characters in the preceding generation is due to their development not from *those characters* but from homologous determinants. Characters of this type are truly determined and all hereditary characters are reducible to this type whether they are exhibited in egg, sperm, embryo or adult.

It is now possible and desirable to define the expressions "inheritance through the cytoplasm" and "inheritance through the chromosomes." The first properly means that the locus of the determiners or representatives of a character is the cytoplasm, and since it is the egg alone which contains any significant amount of cytoplasm, the expression usually means the presence of these determiners in the egg cytoplasm. "Inheritance through the chromosomes" means that the chromatic substance of the nucleus is the locus of determiners, and since the nuclear content of egg and sperm is equivalent this must also mean an equal determinative share by egg and sperm in heredity.

Are now both of these theories compatible with one definition of "determined"? Are they both possible and both necessary?

Loeb has stated the problem and the "compromise" in these words (1916, p. 245):

> Question: "Is the organism nothing but a mosaic of hereditary characters determined essentially by definite elements in the chromosomes; and if this be true what makes a harmonious whole organism out of this kaleidoscopic assortment?"
>
> Answer: ". . . the cytoplasm of the egg is the future embryo in the rough, and the factors of heredity in the sperm only act by impressing the details on the rough block."

Shull's statement is as follows (p. 6):

> The cytoplasm often (perhaps usually) determines the type of cleavage, the early course of development, and in large measure the larval characters, while the adult characteristics are determined by the chromosomes.

Conklin's conclusions are (1915, p. 176):

> There is no doubt that most of the differentiations of the egg cytoplasm have arisen during the ovarian history of the egg and as a result of the interaction of nucleus and cytoplasm; but the fact remains that at the time of fertilization, the hereditary potencies of the two germ cells are not equal. All the early stages of development, including the polarity, symmetry, type of cleavage, and the pattern or relative position of future organs, being foreshadowed in the cytoplasm of the egg cell while only the differentiations of later development are influenced by the sperm. In short the egg cytoplasm fixes the general type of development and the sperm and egg nuclei supply only the details. We are vertebrates because our mothers were vertebrates and produced eggs of the vertebrate pattern—but the color of our skin, eyes and hair . . . were determined by the sperm as well as by the egg from which we came.

The same author has reiterated and somewhat elaborated the same views in an unpublished paper presented before the National Academy of Science in November, 1916.[1]

[1] Since the above was written Conklin's paper (1917) has been published. In its closing paragraphs he modifies materially the view which he had earlier expressed, admitting that cytoplasmic differentiation of the egg-cell probably arises under nuclear influence exerted equally by the egg and the sperm nuclei of the previous generation, the view maintained by the present writer.

The evidence and a criticism of parts of it follows:

1. Shull's Evidence

(*a*) Cases of maternal inheritance. Under this heading Shull places such experiments as those of Correns (1909) on *Mirabilis jalapa* var. *albomaculata*. This plant —the common four-o'clock—has variegated leaves, green and white, the white being due to inhibited development of green in the chromatophores. The amount of green and white varies in different plants and furthermore whole branches may be green while other whole branches may be white. Flowers borne upon green branches, if self-fertilized, give seed that produces only green offspring. Flowers from white branches, if selfed, give seed that produces only whites, which die because they are unable to carry on photosynthesis. Flowers on variegated branches yield offspring some of which are green, some variegated. Crosses among these green, white and variegated plants reveal the fact that the offspring resemble invariably the female parent. White females pollinated by any green or variegated pollen yield only whites which die. Green females pollinated by white or variegated pollen yield only green descendants. The paternal character never reappears in subsequent generations.

Correns has assumed in explaining these remarkable occurrences that a disease transmitted by the cytoplasm of the ovule is the cause of the color differences, inasmuch as the white color in either self condition or as mottling on the green is a pathological condition. The chromosomes are assumed to be immune to this disease. If the disease is caused by a germ (which is very likely) this germ acts only on the chromatophores and may well be passed through the egg like the germs of syphilis and other diseases. If this is the case, true heredity is not involved. The egg has simply acted as the passive bearer of a foreign body. And if the disease is due to a defect in the chromatophores, the case is not very different. The

chromatophores, as Shull says, are probably "autonomous bodies arising only from other autonomous bodies like themselves." On such a view they are simply structures enclosed within the cytoplasm, having a continuity parallel with but independent of the continuity of inherited characters.

(*b*) Shull's next evidence is drawn from experiments resulting in so-called "matrocline hybrids," which he defines as "unequal reciprocal hybrids which resemble the mother more than the father." Under this head he cites the well-known cases of species and genera crosses among echinoderms. He says of the first generation from sea-urchin ♀ × starfish ♂ (Loeb, 1903), and from sea-urchin ♀ × crinoid ♂ (Godlewski, 1906) that the embryos were purely maternal in character. In contrast to this, other observers of species and genera crosses among echinoderms (Morgan, Boveri, Baltzer and Herbst) have described the F_1 embryos as intermediate between the parents wherever there was normal union of maternal and paternal chromatin. Shull emphasizes especially the maternal character of embryos possessing no maternal chromatin. These were produced by Godlewski, by fertilizing enucleated fragments of sea-urchin eggs with crinoid sperm. The larval stages were reported to have been purely of the sea-urchin type. However, Boveri, Bierens de Haans and Herbst have obtained results which showed either the reverse condition, viz., *paternal* embryonic characters, or else intermediate larvæ. Moreover in the cross fertilization of giant sea-urchin eggs possessing twice the normal amounts of cytoplasm and chromatin, the larvæ inclined to the maternal side. That this was not due to the doubled amount of cytoplasm was demonstrated by Boveri, who fertilized *nucleated* half-fragments of normal eggs with sperm of another species and found no paternal inclination from the halved amount of cytoplasm. That the phenomena are due rather to the chromosomes is indicated by the same experimenter's work with dispermic fertilization. When more than one

sperm enters the egg abnormal mitotic figures and abnormal chromatin distribution are directly correlated with abnormalities in the larvæ, although the egg cytoplasm remains constant. Baltzer's fine work on species crosses may also be cited as showing that Loeb's and Godlewski's observations penetrated only part way toward the truth.

As an example, only one of Baltzer's crosses need be cited. When eggs of *Sphærechinus* were fertilized by sperm of *Strongylocentrotus,* the mitotic figures and distribution of chromatin were normal and the larvæ were intermediate, *not* maternal. From the reciprocal cross "matrocline hybrids" (most of them abnormal) did result, *but* their maternal resemblance was not due to cytoplasm. That it was due to irregularities in the chromatin distribution was proved by Baltzer, who followed the history of the maternal and paternal chromosomes in the hybrid embryos. He found that the majority of the paternal chromosomes (15 out of 18) were inactive at the first cleavage. They were extruded from the developing oösperm nucleus, and degenerated. The cells of the hybrid had then 21 chromosomes, 18 maternal and 3 paternal, and their maternal resemblance is easily explicable on these grounds. It is not explicable on any other for no abnormal conditions obtained in either cytoplasm or the surrounding medium.

Herbst repeated the first of Baltzer's crosses (*Sphærechinus* ♀ × *Strongylocentrotus* ♂) but chemically induced parthenogenetic development in the egg before the entrance of the sperm. Thus the ♂ pronucleus was behind at the first division and failed to be incorporated into the nucleus of one of the first daughter cells. One side of the developing hybrid had, then, merely paternal chromosomes, while the other had both maternal and paternal, and in striking sequence to this distribution were embryos which had only paternal characters on one side, while those of the other side were intermediate.

On the evidence thus far, Shull himself has not placed

the maximum of emphasis, and the foregoing criticism has been intended to indicate that its support of the cytoplasmic view has been and may continue to be so minimized as to be non-existent. However, Shull does place much emphasis on some carefully collected evidence of his own. This does not, I believe, support his theory to any greater extent than his quoted cases.

The evidence is briefly as follows: Shull crossed two lines of rotifers which differed in two egg characters—time of hatching of sexual eggs, and the proportions of sexual egs which actually hatched. The eggs of line "A" hatched on the average in 1–2 weeks, about 50 per cent. emerging. Line "B" eggs took 5–6 weeks to hatch and only 5 per cent. emerged. Line "A" females fertilized by line "B" sperm laid eggs which hatched in 1–3 weeks, 50 per cent. emerging. The eggs thus resembled the mother's line in both respects. Line "B" females fertilized by line "A" sperm laid eggs, 30 per cent. of which emerged in 4–5 weeks, resembling more the mother's line than the father's. The reciprocal hybrids are thus very unequal, says Shull, and since in crossing parents which differ by Mendelian, chromosome-determined characters, the resulting reciprocal hybrids are equal, the characters under observation are non-Mendelian and determined in the cytoplasm of the egg.

But it is to be objected that in reality these hybrid eggs of the first generation are not hybrid in these characters at all. The characters are egg characters and as such can be exhibited only when the hybrid zygote produces its eggs, not when the hybrid zygote is formed. The expression of the character is thus delayed until the hatching of eggs laid by these F_1 zygotes. And Shull's data shows this to be the case.

> But when new lines were obtained from these hybrid (F_1) eggs, and these lines produced sexual eggs of their own, the two reciprocal hybrid lines were *fully equal.*

Now the usual occurrence is observed, viz., the reciprocal hybrids are equal and the contributions to the character

by ♂ and ♀ are proved to be also equal. Shull's contention for the participation of the egg cytoplasm rests entirely on the maternal character of the first egg-generation. These eggs were matured, since the mother was homozygous in the characters, under the influence of the *like* chromatic contributions of her parents; the hybrid mother matured hers under the influence of the *unlike* chromatic contributions of her parents and showed the participation of her paternal chromosomes only in the *behavior* of her eggs. The peculiarities of the case lie not in that we are dealing with a cytoplasmically determined character, as Shull contends, but in (1) the fact that the characters are exhibited only by females; (2) in the fact that the characters are egg-characters, which places segregation one generation farther away from the original cross.

The case is quite analogous to the case of the inheritance of red pericarp color in corn, which, although a "maternal character," was shown to Mendelize by Lock ('06). It is also comparable to the egg-character "unibivoltinism" in silk moths, which Castle ('10) proved from Miss McCracken's data to be a Mendelian character. These cases will be discussed more fully in later paragraphs.

Shull's conclusion that cytoplasm determines egg and larval characters is, I believe, unnecessary. It has been shown that characters exhibited only by females and only in the egg may be equally determined both in the egg and in the sperm. The sperm contribution being predominantly chromatic, and the chromosomes being the accepted carriers of the determiners of other characters, it is to be concluded that the determiners for the characters investigated by Shull are also to be sought in the chromosomes.

Loeb, in his book (1916), has given considerable space to this question, closely following the earlier treatment by Conklin. His conclusion which has been stated and the evidence on which it rests may be briefly criticized by the

addition of further evidence which warrants a changed interpretation of certain facts.

Loeb first calls attention to the exclusively maternal character of the early development of enucleated fragments of eggs when fertilized by sperm of a different species. Such evidence has already been treated above (p. 5).

His second claim—that the *rate* of cell division and development is determined only by the egg cytoplasm—warrants further consideration. An egg from a line in which segmentation of the egg takes place eight hours after fertilization was fertilized by sperm from a line in which segmentation begins in 30 minutes. The rate of these cross-fertilized eggs was 8 hours, like the mother's line.

The careful and long continued work of Newman ('14) has, on the other hand, shown that the entrance of sperm of a different species does materially alter the rate of development.

> In both reciprocal crosses between *Fundulus heteroclitus* and *F. majalis* the rate of development of the hybrids was intermediate between that of the two parent species. This was true of cleavage rate, rate of germ-ring formation, etc.

In the cross *F. heteroclitus* $\times$ *F. diaphanus* "both reciprocal crosses have a higher rate than the pure bred strain. Similarly, when we make reciprocal crosses between *Cypronodon* and any species of *Fundulus* we find a marked retardation in developmental rate in both crosses. . . ." It is of the greatest significance that in all three cases the results of reciprocal crosses were equal. Either both were intermediate, both were accelerated or both were retarded regardless of which species was used as the egg-parent. In the face of such evidence, a theory of exclusive control of the egg over early development is untenable.

Newman's fundulus hybrids, while demonstrating the conclusion just stated, do not form critical evidence for determining the action in heredity of such rate-characters because only the F_1 generation is known. Another series

of experiments on a hatching time character has, however, been carried through the F_3 generation and as an illustration such a case may be cited in detail. It consists of Miss McCracken's (1909) experiments with silk moths. Castle later (1910) called attention to some facts in her data which indicated that although a female-exhibited character and confined to the egg in its expression, it nevertheless gave evidence of Mendelizing in crosses. Toyama ('12) concluded that dominance was present, and both of the latter investigators agreed that the males, although unable to exhibit the character, gave evidence by their genetic behavior of having an equal determinative influence with the females. The data follows:

Silk moths lay one batch of eggs, always in the spring. The eggs of some batches hatch out immediately, producing another brood of larvæ and moths in that season. The parents of such batches of eggs are hence known as bivoltins. The eggs of other batches do not hatch for twelve months, and since in this way there is but one brood or flight each season, the parents of such eggs are known as univoltins. If a univoltin female is crossed with a bivoltin male, the spring batch is laid as usual and hatches in 12 months. This is just what would have occurred if the mother had been fertilized by a male of her own sort. When these eggs hatch, a hybrid brood emerges which lay their egg batches immediately but the univoltin character is again exhibited in that all of these eggs are of the 12 months type. But, these eggs now differ among themselves as is shown by the behavior of the zygotes which emerge from them. Some of these females are bivoltin, laying eggs which develop immediately, while others are univoltin, laying eggs which hatch the following spring. The expression of the paternal contribution is delayed, but its activity in determining the time of hatching is quite apparent.

The inheritance of red pericarp color in corn follows exactly the same course as that outlined above, with red dominant over white. The F_2 embryos must be *raised*

before the segregation of pericarp color among them can be seen, for it is exhibited only in the seed coats. The conclusions follow: (1) The egg and all its determinative content is produced under the double influence of the sperm and egg chromatin contributions which united to form the producing zygote. (2) Hybridization experiments with egg characters, to be critical, must be carried as far the F_3 generation. (3) In all experiments which I have seen reported, in which this condition obtained, the influence of the sperm on the characters in question has been observed.

Loeb's evidence, however, introduces also crosses between *Strongylocentrotus purpuratus* and *S. franciscanus,* and the statement is made that the development of the hybrid up to the formation of the skeleton resembles exclusively the development of the mother's species. But Loeb also finds that the cross-barring in the spicules of *purpuratus* behaves as a dominant character in *reciprocal crosses.* He assigns this character to a factor, which he imagines to be a ferment or enzyme. This statement follows: "Since the pure *purpuratus* has two determiners for the development of the cross-bars, and the hybrids only one, the pure *purpuratus* should have twice the enzyme and develop twice as fast"—and it did. He provides here not only evidence that avowed chromosome characters do affect the *rate* of development, but even furnishes an enzymatic mechanism by which they may do it. And yet soon after the above quotation, we read:

> We can therefore be tolerably sure that wherever we deal with a hereditary factor which is determined by the egg alone, the cytoplasm of the latter is partly or exclusively responsible for the result. We have already mentioned that *rate of segmentation* is such a character.

The whole case of the supporters of any theory which views the cytoplasm as determinative rests on either their refusal to go back and inquire the source of this cytoplasm, or on their refusal to give due emphasis to the source, even though they recognize it. Conklin recognizes

the double influence which is exerted on the developing egg better than any of the others who have adopted his "compromise theory." He admits that "most of the differentiations of the cytoplasm have arisen during the ovarian history of the egg and as a result of the interaction of nucleus and cytoplasm." He has demonstrated better than any other one man how complex and definite these differentiations in the egg cytoplasm are. All will agree with him when he says that they "foreshadow" the future organism. But "cytoplasmic organization, while affording the immediate conditions of development, *is itself a result of the nature of the nuclear substance* which represents by its inherent composition the totality of heritable potence." These last are the words of E. B. Wilson (1895, p. 25), although he has translated and adapted them from an earlier paper by Driesch. They represent the opinion of Wilson and of Driesch in full accord. "The nuclear substance" referred to was even then known to contain equality of maternal and paternal chromatin.

Wilson himself had been able to demonstrate that the structure of the cytoplasm in sea-urchin eggs was acquired during ovarian life, and on the basis of this and of a considerable body of similar evidence he was able to conclude quite definitely:

> That a preorganization of the cytoplasm can not be regarded as the primary factor in heredity is conclusively proved by the old argument based on inheritance from the father through the sperm nucleus.

The only link which is needed to make the chain complete is some substantial body of evidence, demonstrating the effect and the mechanism of action of the nucleus on the cytoplasm. This, it must be admitted, has not been entirely filled. Nägeli, to be sure, held a theory of a dynamic effect of the nuclear idioplasm on the cytoplasm, while Driesch contended that the mechanism was chemical. The nucleus, in his opinion, exercised its governance by means of ferments or enzymes. There are facts in

development which point to effects of the nucleus even on the visible differentiation of the egg before fertilization. In the sea-urchin, for instance, this differentiation is preceded by the absorption into the nucleus of part of the fluid content of the cytoplasm, altering the chemical composition of the latter and greatly increasing the bulk of the nucleus. The membrane of this enlarged nucleus then dissolves and part of its contents by their color may be traced to a clear cap of fluid which later gives rise to the skeleton of the echinoderm. Such absorptions and minglings probably play a large part in the reactions of nucleus and cytoplasm, either at the successive disappearances of the nuclear membrane during mitosis or through that membrane.

Nucleus and cytoplasm may certainly be regarded as forming a reaction system analogous to that which might exist between a series of chemical substances (Jennings, 1914). The cytoplasm in turn is linked with the extracellular milieu in a quite comparable way and forms the intermediary between the nucleus and the exterior.

Evidence on this interaction is accumulating. As an example I may quote the work of Cameron and Gladstone on cells other than ovarian, and, to be sure, by the static, histological method. But they have observed fine preparations and have concluded that the cytoplasm is visibly differentiated into two grades of endoplasm. The first is next to the nucleus, is clear and refractive. This is the nascent material of the cell and is the first visible stage in the genesis of protoplasm. It is, they postulate, a derivative of the nucleus itself, and to the nucleus is ascribed the final elaboration of nutritive material which has been ingested by the cytoplasm. This nascent endoplasm they conceive to be the active material of the cell grading into passivity toward the cell periphery, through the maturer endoplasm and the ectoplasm.

Whatever relations may exist between the two, the fact remains that the cytoplasm is necessary. Without it the nucleus, deprived of its milieu, can not live, and develop-

ment can not take place. The investigation of the finer physiological reactions which take place between the nucleus and cytoplasm is badly needed, and the restatement of them in terms of physics and chemistry. Such evidence as is available indicates that the importance of the cytoplasm is, in the main, subordinate to that of the nucleus.

The evidence from egg-characters, it might be noted in conclusion, is one-sided. I have no doubt that if sperm-characters were to be studied as intensively as egg-characters have been (which has not been the case due to the microscopic size of most sperms) the differential characters in the sperm would be found to behave in heredity like the differential characters of eggs, and would be determined as largely by the egg nucleus as by the nucleus of the sperm of the preceding generation.

Conclusions

Direct continuity of substances in the cytoplasm is not a method of heredity. It simply provides for the autonomous proliferation of materials with no determinative significance. No compromise, then, is possible between the two views outlined as "cytoplasmic" and "chromosome" theories of heredity. The first is non determinative; the second is the primarily effective method of heredity and of development. The working of the effective method is known for heredity, if heredity be properly only concerned with the way in which the hereditary factors are distributed in the germ cells. For development, its mechanism is but grossly known, but we have learned enough of the determinative effect of the nucleus and of the possibilities for interaction between cytoplasm and nucleus to foster a suspicion that one day the governance of the chromosomes over development will be explained in physical-chemical terms.

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